FINAL REPORT

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Effects of Lysergic Acid and Its Derivatives on Rhinencephalic Electrograms

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Heath et al have reported subcortical paroxysmal activity recorded from the septal and hippocampal regions in patients suffering from schizophrenia (1,2). In a continuation of these studies, Heath and associates studied the rhinencephalic electrograms of humans under the influence of LSD and mescaline (3). They reported that paroxysmal activity induced in the hippocampal, amygdaloid and septal regions seemed to correlate with overt expressions of psychotic behavior. It seemed relevant then to check these findings by giving lysergic acid derivatives with varying degrees of psychotogenic effects to animals with chronically implanted subcortical electrodes in these rhinencephalic structures. It was felt that such studies might elucidate whether this paroxysmal hypersynchronous activity in the rhinencephalic region was a spurious or an essential neurophysiologic mechanism in individuals demonstrating psychotic behavior -- whether spontaneous or pharmacologically induced. It was hoped that a gross correlation could be made between the extent of hypersynchronous activity in the rhinencephalic structure and the overt behavior of the macaca mulatta monkey, and despite species difference there might be a gross correlation between this rhinencephalic activity and the psychotogenic effects in man of various lysergic acid derivatives as established by Harris Isbell (4). If this would be the case, then it would offer a screening device for determining not only psychotogenic activity but also as previous studies

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indicate effective tranquillizers alter this paroxysmal activity (3), it might offer effective means for screening tranquillizing drugs.

Method

Electrodes were placed in the macaca mulatta monkey by Heath's associates* using a method previously described by Heath and associates for animals and humans (1,5,6). This method employed the stereotaxic instrument with an air study of each animal and the x-ray forming an individual map since the stereotaxic atlas is apt to be unreliable for these studies. All areas into which electrodes were implanted are close to the ventricular system. Cortical electrodes were placed over pre-determined areas of the cortex (Fig. 1). The desired locations for the electrodes in terms of the coordinates of Olszewski's atlas (7) were as follows:

anterior septum	A.P. +24.5	Depth + 8.5	Lat. midline
posterior septum	A.P. +20.5	Depth + 8.5	Lat. 1.0
caudate	A.P. +22.5	Depth +11	Lat. 5
anterior hippocampus	A.P. + 9	Depth - 6	Lat. 14
posterior hippocampus	A.P. + 0.3	Depth + 1.52	Lat. 14

After histological studies it was felt that this posterior hippocampus location was too far posterior, so that in later monkeys it was changed to:

A.P. + 3.0 Depth + 8.5 Lat. 11

At the time of the animals' death, the brains were fixed, sectioned, stained and the placement determined histologically (Fig. 2, 3, and 4). The animals were studied over a period of from 1 to 3 months with 3 to 20 studies done on each animal. No drug studies were done without an intervening 48

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* Charles Fontana and Owen Foss, Dr. Heath's technicians.

hours or until baseline had returned to normal. The animals remained free in their cages except during the experiments when they were partially restrained in a chair with a collar which prevented them from reaching the electrode wires. A crude behavioral rating was kept on the animals which included both autonomic and voluntary activity such as dilatation of pupils, flushing, temperature of extremities, vomiting, urination, defecation, alertness, defensiveness, resistance, posturing, agitation, vocalization, and unresponsiveness. Aside from the subcortical electrodes mentioned above, frontal and parietal-occipital cortical recordings were made. The standard 8-channel Grass machine with both monopolar and bipolar recording methods was used. When monopolar recordings were made, the caudate electrode was used as reference because of its relative inactivity.

After the electrograms had returned to normal following operation, each animal was given d-LSD-25 in doses ranging from 70-110 gamma per kilo, depending on the level that would elicit an unequivocal paroxysmal hypersynchronous activity in the rhinencephalic structures. This was used as a crude measure of the animal's susceptibility to the lysergic acid derivatives and from then on the other drugs' doses were first determined on the basis of relative psychotogenic activity as compared with d-LSD-25. For example, if the animal needed 100 gamma per kilo d-LSD to elicit appropriate rhinencephalic activity and the psychotogenic effect of the lysergic acid derivative, for instance MLD-41 was 40% of d-LSD-25 as determined by Isbell (4), 250 gamma per kilo of the MLD-41 would be given. However, as the experiment progressed, we found that we had to modify such a rigid determination of dose.

d-LSD-25 (d-lysergic acid diethylamide)

This drug was given in doses of 70 to 110 gamma per kilo on 7 different occasions. In 2 instances there was dramatic catatonic-like behavior, and in both these instances there was 7 to 20/second paroxysmal hypersynchronous activity in the hippocampus and septal regions. This was also reflected in the cortical regions (Fig. 5 a & b). In 2 other instances, the catatonic-like behavior was slight but definite, and again there was 7/second hypersynchronous activity in the hippocampus and septal region. On 3 other occasions, the animal was either drowsy or agitated. In these instances, there was paroxysmal activity in the cortical and the hippocampal regions, but it was absent in the septum. From these studies on d-LSD, it would seem that the crucial location of electrographic activity is the septal region -- the more marked the changes in this region, the more marked the behavioral changes in the animal, particularly catatonic-like behavior. *

One monkey received the same dose of d-LSD-25 one month apart and showed a different response on the 2 occasions. In the first instance, there was immediate passive behavior with no biting, no resistance and definite posturing, which we designate catatonic-like behavior. This was maximal for a period of one hour and present to a slight degree at the end of 2 hours. In this experiment there was generalized slowing with bursts of 13/second activity in both the cortical and hippocampal regions and occasional low amplitude activity of a similar type in the anterior septal region. In the record a month later there was the paroxysmal activity in

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* By catatonic behavior, we mean the animal is generally unresponsive although obviously awake and in some contact with his surroundings; that is, he will follow the examiner with his eyes. However, he does not show the usual defensive reaction to blowing in the face, touching the mouth, nor does he struggle or resist when his limbs are moved. On moving the limbs, one notes to a greater or lesser extent the waxy flexibility seen in patients, and there is some tendency to posture -- that is, for the animal to maintain an uncomfortable, unnatural position (molding).

the 7-15/second range in the hippocampal region and paroxysmal 15/second activity in the occipital cortex, but this time none in the septal region. Although the animal was passive and showed no biting or resistance, there was no obvious posturing. Giving the animal the same dose the following day, there were no behavioral changes and only rare paroxysmal activity similar to the previous day's recording. This dramatically demonstrates the rapid tolerance the animal develops to d-LSD-25 (Fig. 6 a, b & c) both clinically and electrophysiologically.

ALD-52 (d-l-acetyl-lysergic acid diethylamide)

This drug was given in doses from 140 to 500 gamma per kilo on 3 occasions. Isbell reports this drug shows 13% of the pyretogenic activity, 200% of the antiserotonin activity, and 100% of the psychotomimetic activity of d-LSD-25 (4). Three studies were done. In all three instances the animal became agitated. With this agitation was 12 to 15/second paroxysmal activity in the frontal, hippocampal and occasionally parietal leads with only minimal reflection in the septal leads (Fig. 7 a & b). No catatonic-like behavior was observed.

MLD-41 (d-l methyl-lysergic acid diethylamide)

This drug was given in doses ranging from 50 to 200 gamma per kilo on 2 occasions. The drug shows 5% of the pyretogenic, 370% antiserotonin effect, and 40% psychotomimetic effect of d-LSD-25 (4). In both instances the animal became quite placid again showing 12 to 15/second paroxysmal activity in frontal and hippocampal regions with possible involvement of the septal and/or caudate region (Fig. 8). This placid behavior could have been minimal catatonic-like behavior.

However, in the animals that showed dramatic behavioral changes there also was striking change in the septum. However, MM-2 (Fig. 10) was the one example of an animal that showed definite changes of a paroxysmal hypersynchronous nature in the septal region and did not show the catatonic-like behavior defined above. Instead, the animal was extremely agitated and fearful, defecated frequently, showed excessive defensive reactions and appeared as though he was responding to hallucinations.

LPD (d-lysergic acid pyrrolidide)

This drug was given at a dose level of 40 gamma per kilo. It has 10% of the pyretogenic effect, 5% antiserotonin effect, and 10% psychotomimetic effect of d-LSD-25 (4). Two studies were done. One animal showed dramatic flaccid response for short periods which might have been an early catatonic-like response although there was no definite evidence of posturing. This flaccid behavior alternated with short periods of extreme excitability. This animal had slow paroxysmal activity of 6/second and spikes appearing in the frontal, parietal, septal and hippocampal regions with generalized slow background activity (Fig. 11). Again this is another example of dramatic EEG changes and behavioral changes in a drug that is reported to have little psychotomimetic effect on humans. In the second animal receiving the same dose, there was slow, high amplitude paroxysmal activity immediately appearing in the cortical and hippocampal regions, but not in the septum. In this instance, however, the animal became much less defensive, did not bite, but continued to resist and showed no evidence of posturing.

l-LSD-25 (l-lysergic acid diethylamide)

This drug was given in dose ranges of 300 to 450 gamma per kilo on

3 different occasions. It is allegedly inactive and this seemed to be confirmed by the EEG findings, there being no changes and no behavioral effects.

BOL (d-2-brom lysergic acid diethylamide)

This drug was given in dose ranges of 110-175 gamma per kilo. The drug supposedly has 5% of the pyretogenic effect, 103% antiserotonin effect and 0% psychotomimetic effect of d-LSD-25 (4). In the 3 studies done, there was no obvious behavioral change. For an unusual reaction to the administration of BOL, see the paragraph describing the 5-HT + PIH administration.

UML (1-methyl-D-lysergic acid butanolamide)

This drug has 2 to 5 times the serotonin antagonistic effect of d-LSD-25 but has no psychotogenic effect although in human volunteers it does produce fatigue, some impairment of mental concentration and slight euphoria. In one experiment giving 80 gamma per kilo, there were no behavioral changes nor electrographic effects.

Mescaline

An unrelated compound chemically, but one with dramatic psychotomimetic activity, namely mescaline, was also given in 5 studies with a dose range of 9 to 80 milligrams per kilo. One of the studies was equivocal. However, a second showed definite catatonic behavior with 6 to 8/second paroxysmal activity in the cortical, septal, and hippocampal regions (Fig. 12 a, b & c). Immediately after the injection of mescaline, there was a dramatic slowing in all leads, but particularly in the cortical area. This was next followed by fast activity in the 40/second range which looked like muscle artifact.

However, there was a slight reflection of this in the subcortical leads. Studies on other monkeys showed this activity in a somewhat slower range -- that is, 20 to 30/second range -- so that it was felt that this was not an artifact, but legitimate electrical activity coming from the subcortical as well as the cortical areas. The third animal became quite lethargic and then developed convulsions. Spiking activity occurred in the frontal, septal and hippocampal regions, ultimately to be replaced by generalized seizural activity. On the two other occasions although the animal received relatively high doses of 40 to 80 milligrams per kilo and showed some behavioral changes in terms of rolling eyes, immediately reduced defensiveness, no biting or resistance, there was no evidence of catatonic-like behavior and in these instances no evidence of paroxysmal activity in the septal region although the spindle activity in the 20 to 40/second range occurred in the frontal cortex.

Serotonin Effect

Because of the controversial question of the relationship between the d-LSD psychotogenic effect and its antiserotonin effect (recently reviewed by Page) (8), it was felt worthwhile to see what effect a combination of a monoamineoxidase inhibitor -- in this instance phenylisopropylhydrosine (Lakeside JB 516) -- and the serotonin precursor 5-hydroxytryptophane which, unlike serotonin itself crosses the blood-brain barrier, might have on the subcortical electrograms. Four studies were made with PIH at 5 milligrams per kilo. In two of these there was no change while in the third there was some agitation with 15 to 25/second spindle activity in the fronto-parietal region. In two instance, 5-hydroxytryptophane was given in dose ranges from 10 to 20 milligrams per kilo. In one instance there was

generalized slowing while in the other instance no change, nor were there any marked behavioral changes. However, in two studies where the 5-hydroxytryptophane and PIH were combined in the dose levels mentioned above and given simultaneously, the animals were slightly retarded and did show, besides slow delta activity in all leads but predominantly in the cortical area, spike and slow wave in the septal and hippocampal regions (Fig. 13).

There is one other interesting finding which should be mentioned. That is, when MM-4 was given BOL 11 days after the above combination, there was a decided difference in the response to this drug. That is, within 10 seconds, there were bursts of high amplitude, sharp 10-15/second activity in the frontal-hippocampal region very similar to barbiturate spindles. The amplitude was greater than 200 microvolts at the height of the reaction, and was almost continuous at that time (Fig. 14 a, b & c). However, a repeat study on a different monkey failed to duplicate this particular finding.

Discussion

These studies on animals like those previously reported in humans under the influence of LSD and mescaline (3) lend further support to Heath's idea that paroxysmal activity in the rhinencephalic structures, particularly the septal region, grossly correlates with disturbed probably psychotic behavior (1, 2, 6, 9, 10). For instance, such drugs that are known not to have any psychotogenic effect such as l-LSD-25 and BOL did not give this paroxysmal hypersynchronous activity in the rhinencephalic structures, nor did the animals that received these drugs show any noticeable behavioral changes. On the other hand, the electrophysiologic response seems to be more than a spurious correlate to the pharmacologic actions of drugs with

chemical structures related to lysergic acid, inasmuch as mescaline also induces this paroxysmal hypersynchronous activity in the rhinencephalic structures when it induces marked behavioral changes. However, the initial slowing and the fast spindle effect seems more characteristic of mescaline than any of the lysergic acid derivatives. There seems to be no correlation between the rhinencephalic paroxysmal hypersynchronous activity and the reported psychotogenic, pyrogenic and antiserotonin effect of these drugs in human beings. The reason for this remains a moot point. It could reflect a species difference or it may reflect a need for further studies in humans as many of these lysergic acid derivatives have had only limited clinical investigation. Attempting electrographic correlations in animals with other physiologic responses, particularly autonomic responses, might also lend further information, but for technical reasons this was rejected in this particular experiment. Nevertheless, it would seem that screening drugs for their ability to elicit subcortical paroxysmal hypersynchronous activity in rhinencephalic structures would be a reasonably reliable method for determining the psychotogenic activity of drugs or biological preparations. This has already been reported by Heath et al for taraxein (2, 7).

Summary

Six macaca mulatta monkeys had chronically implanted electrodes over the frontal and occipital cortex, and in the septal, caudate, and hippocampal regions. Thirty-two studies were done on such lysergic acid derivatives as d-LSD-25, ALD-52, MLD-41, LSM, DAM, IPD, l-LSD-25, BOL and UML to determine possible correlations between the psychotogenic effect of these drugs and the effect on the subcortical electrogram. No correlation was found between the pyretogenic, antiserotonin or psychotogenic effect as found by Isbell

studying these same drugs on humans. However, there did appear to be a good correlation between the behavioral effect in monkeys (the appearance of disturbed catatonic-like or agitated behavior) and rhinencephalic paroxysmal hypersynchronous activity, particularly in the septal region. Five studies with mescaline also revealed a similar correlation. Studies on a combination of monoamine oxidase inhibitor (phenylisopropylhydrosine) and 5-hydroxytryptophane, a serotonin precursor which crosses the blood-brain barrier, showed minimal effect in the subcortical areas as well as minimal behavioral changes. It would seem that even taking into account species differences, rhinencephalic paroxysmal hypersynchronous abnormality is a good indication of psychotogenic effects of a drug.

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Figure 1

MM-4 in stereotaxic instrument with implanted electrodes from anterior to posterior as follows: frontal cortex, anterior septum, caudate, posterior septum, anterior hippocampus, posterior hippocampus, posterior cortex.

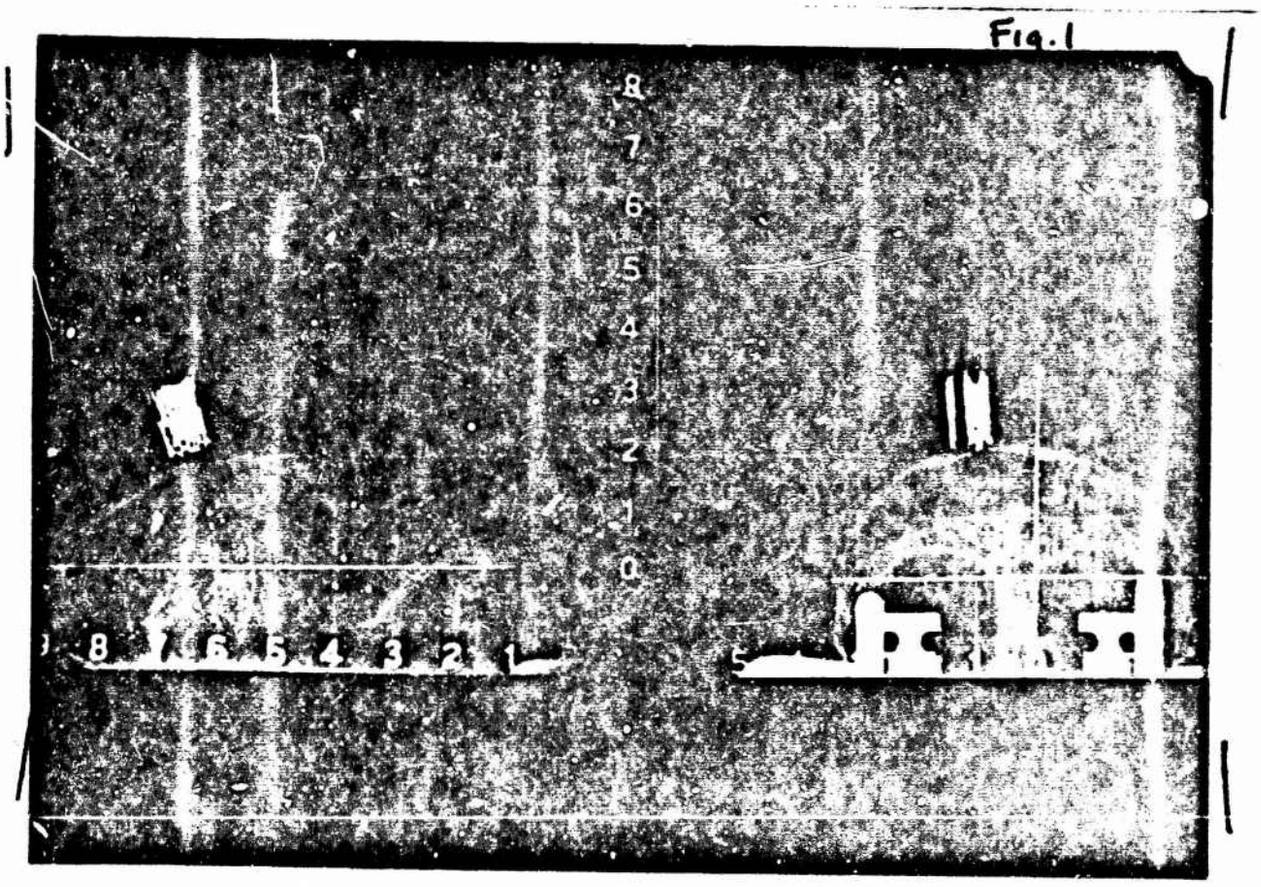


Figure 2

Electrode tract into posterior septal region.

Fig. 2

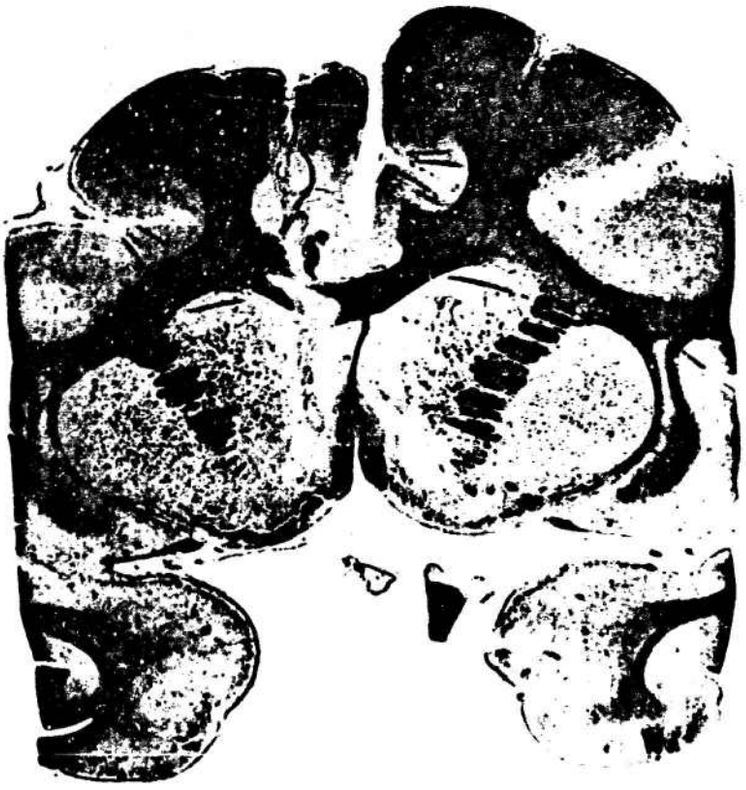


Figure 3

Electrode tract into the caudate nucleus.

Figure 4

Electrode tract into the anterior hippocampal region.

Fig. 4

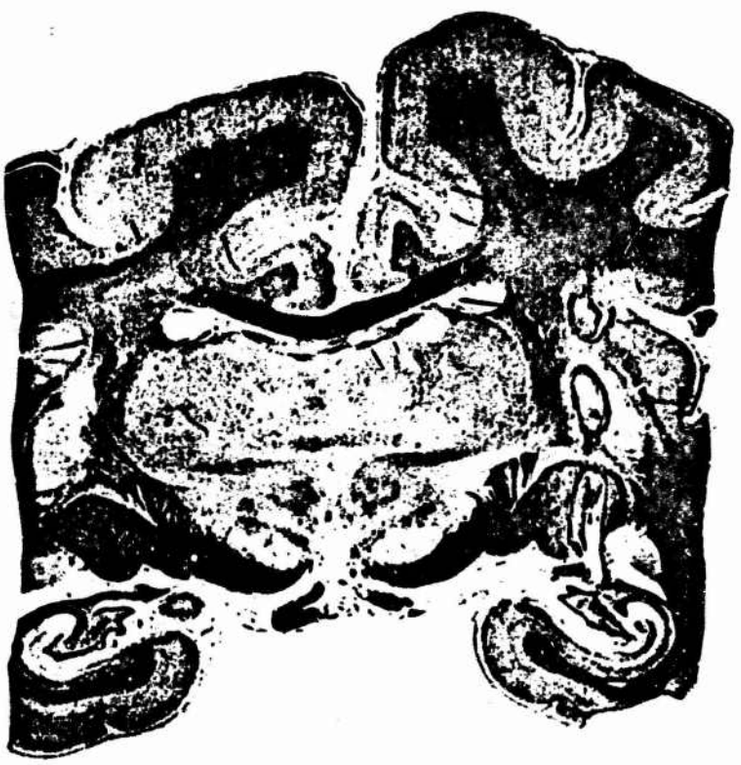
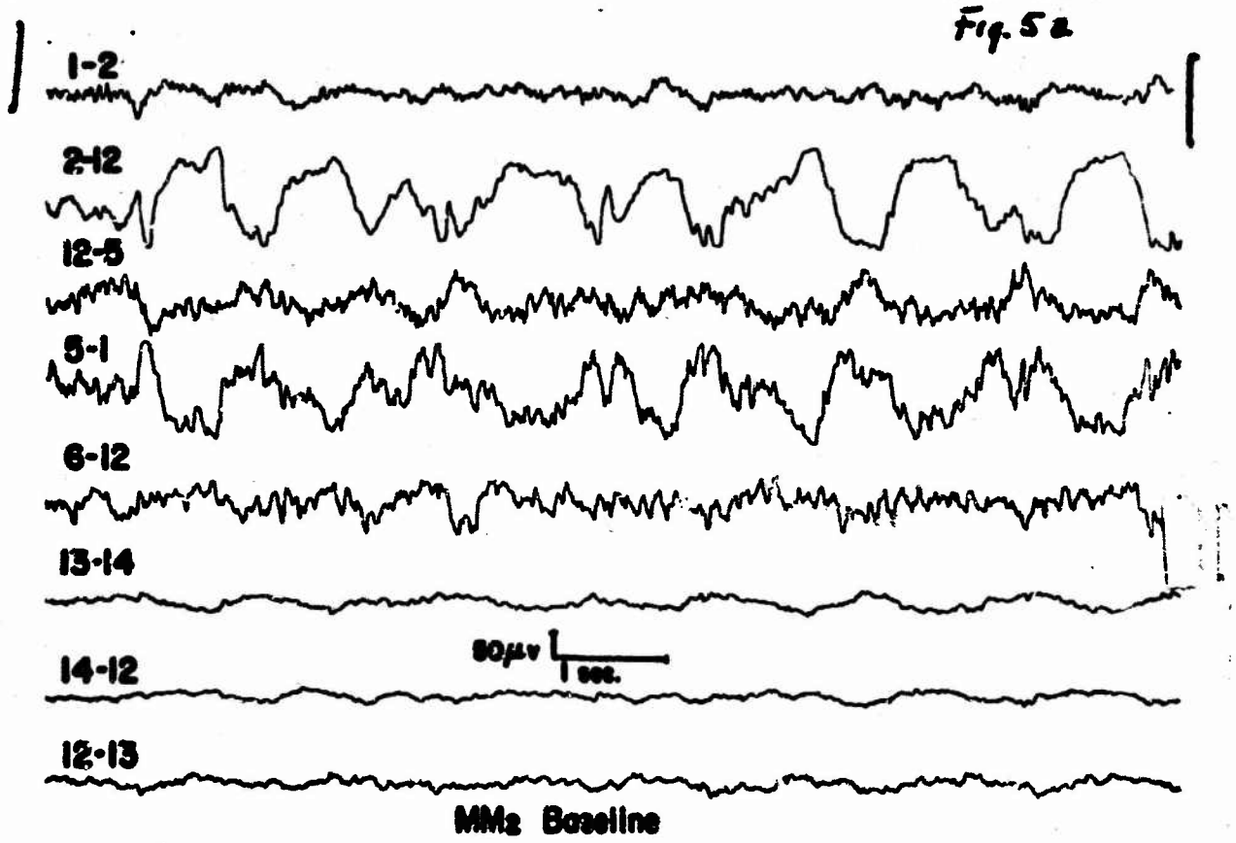


Figure 5 a & b

See text for discussion. (Numerical key for this figure and all others is as follows.)

1. Frontal cortex
2. Parietal cortex
5. Anterior hippocampus
6. Posterior hippocampus
12. Caudate
13. Anterior septum
14. Posterior septum



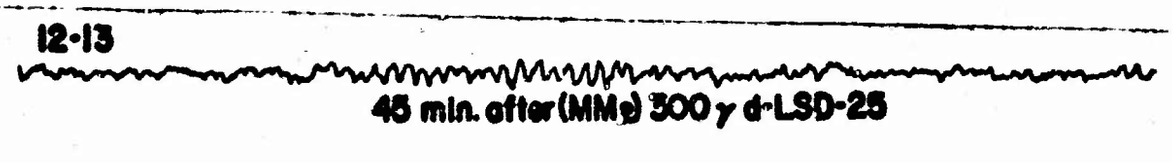
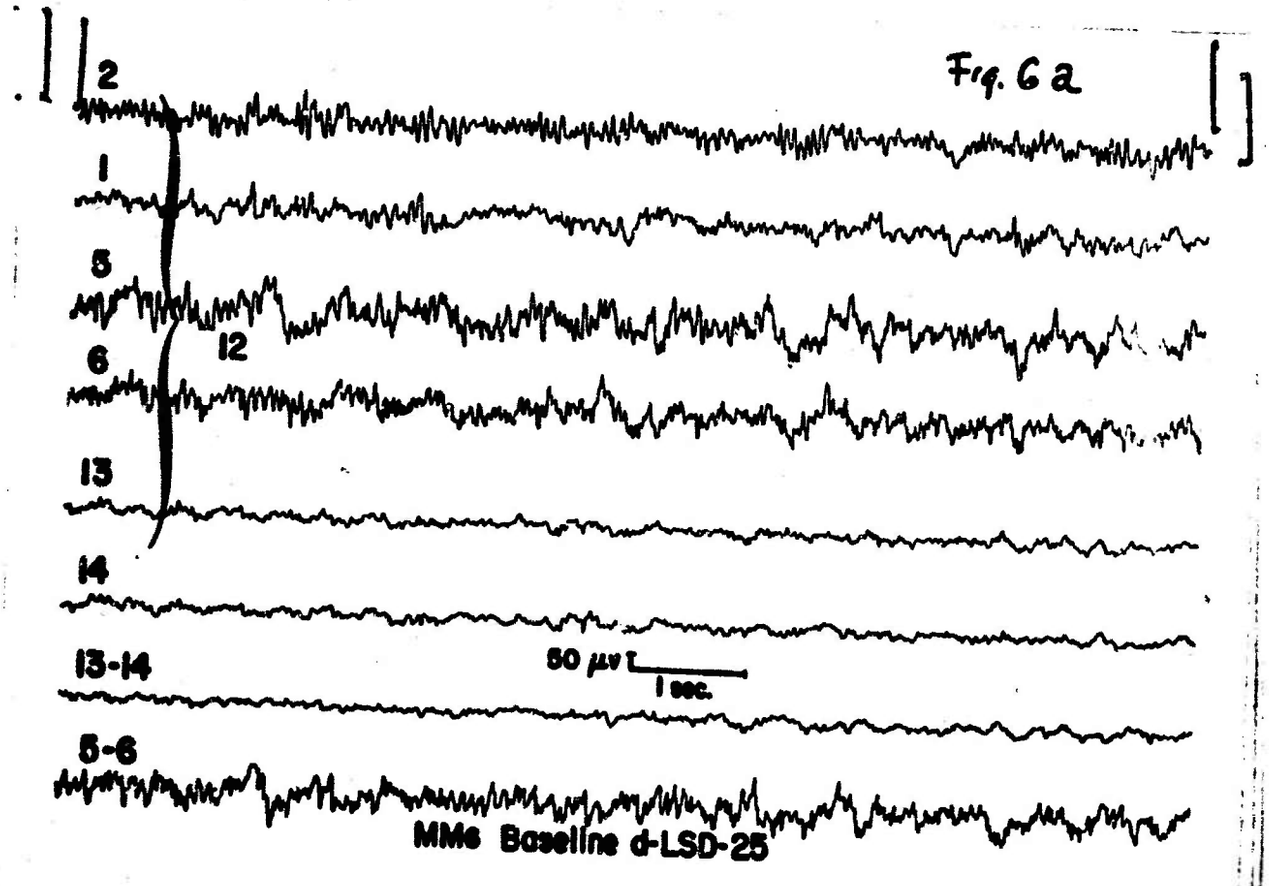


Figure 6 a, b & c

No paroxysmal hypersynchronous activity in septal region and no catatonic like behavior. Comparison of 6b and 6c shows that electrographic tolerance parallels clinical tolerance to d-LSD-25.



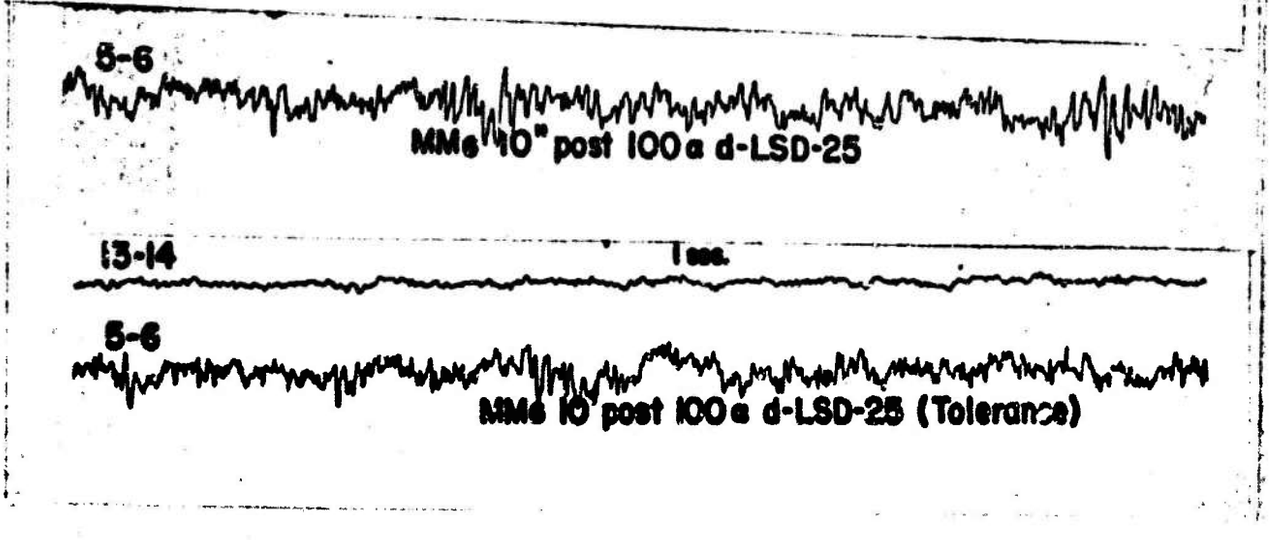


Figure 7 a & b

See text for explanation.

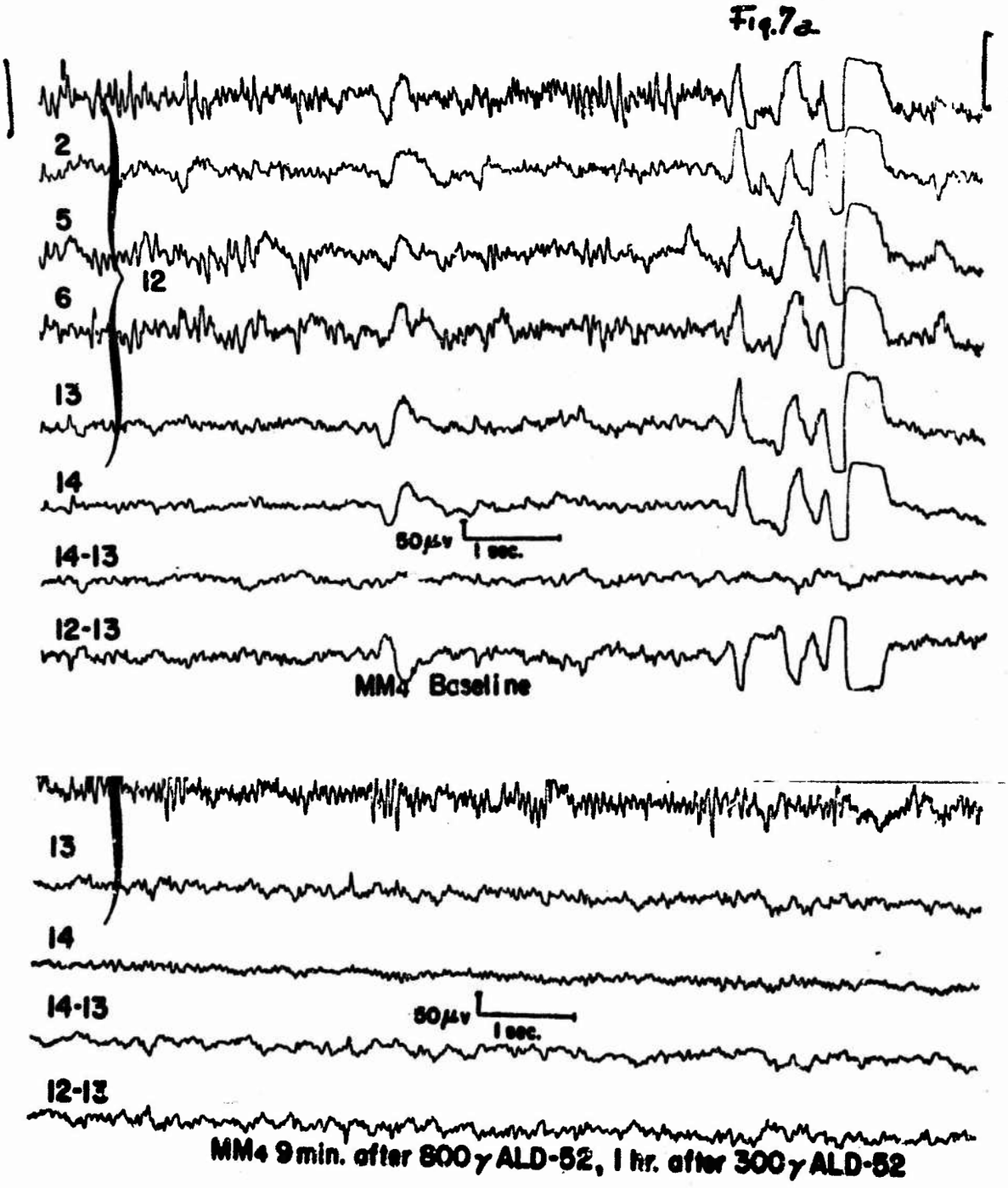


Figure 8

For baseline see Figure 7a.

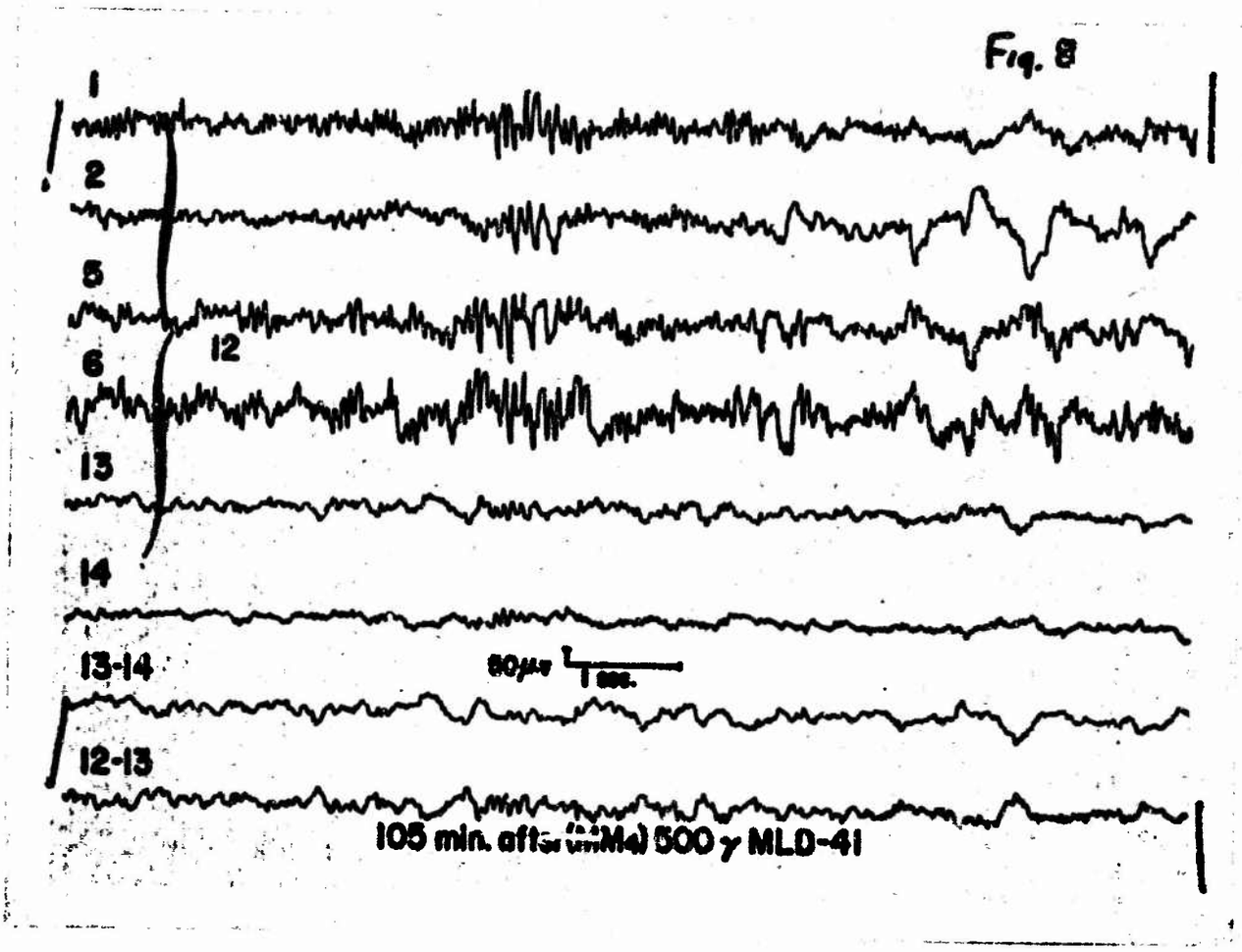


Figure 9 a & b

For baseline see Figure 7a.

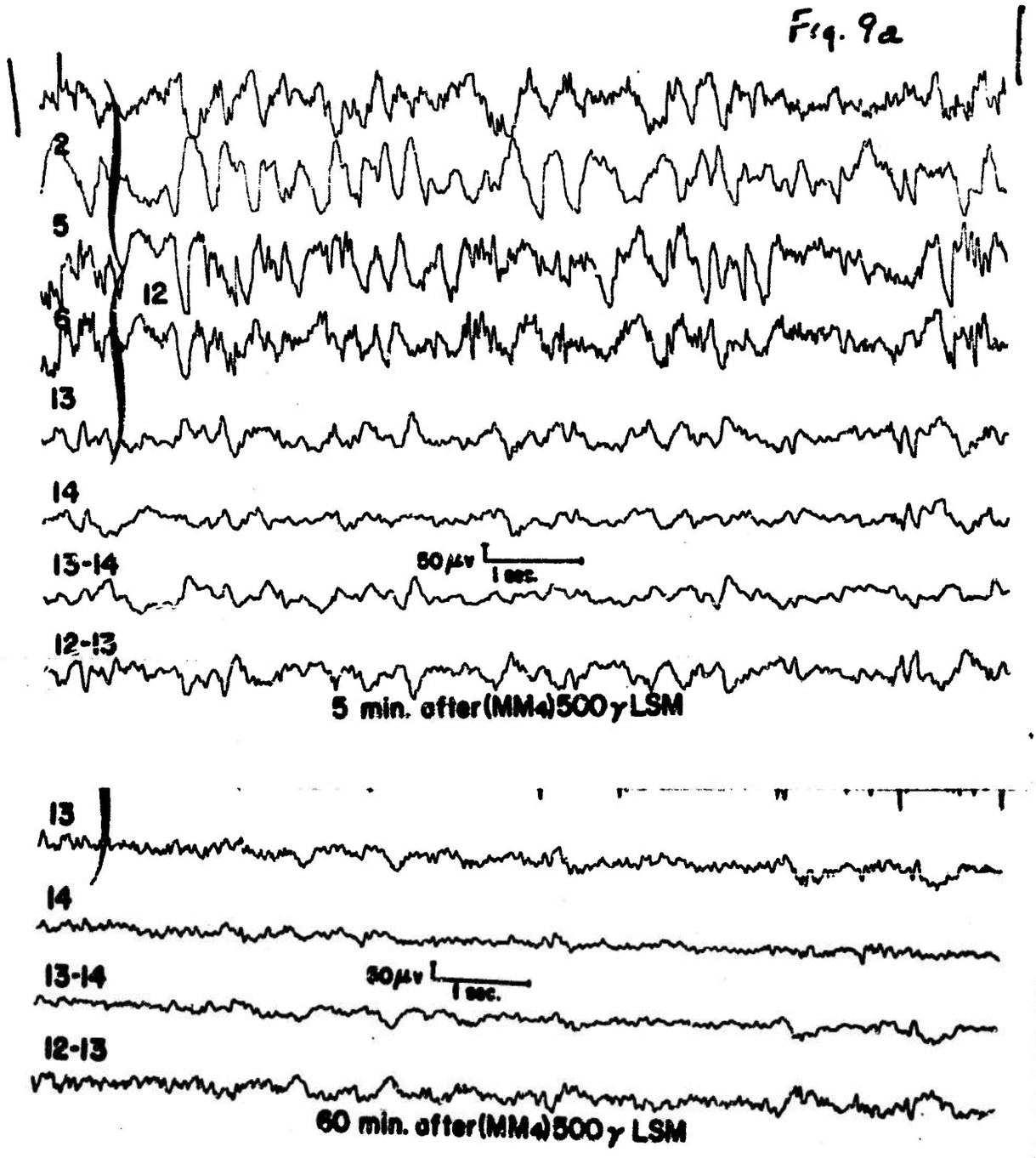


Figure 10

For baseline see Figure 13a.

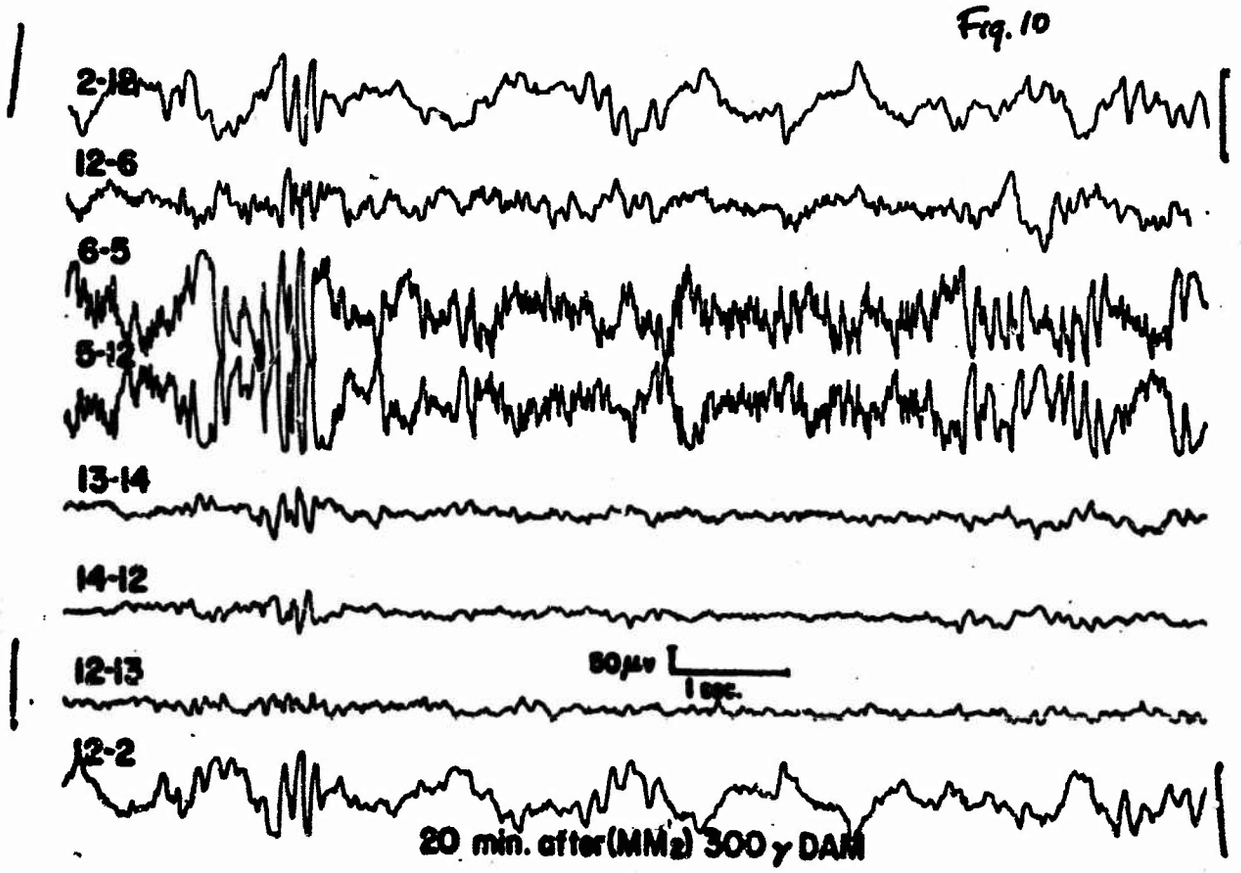


Figure 11

For baseline see Figure 7a.

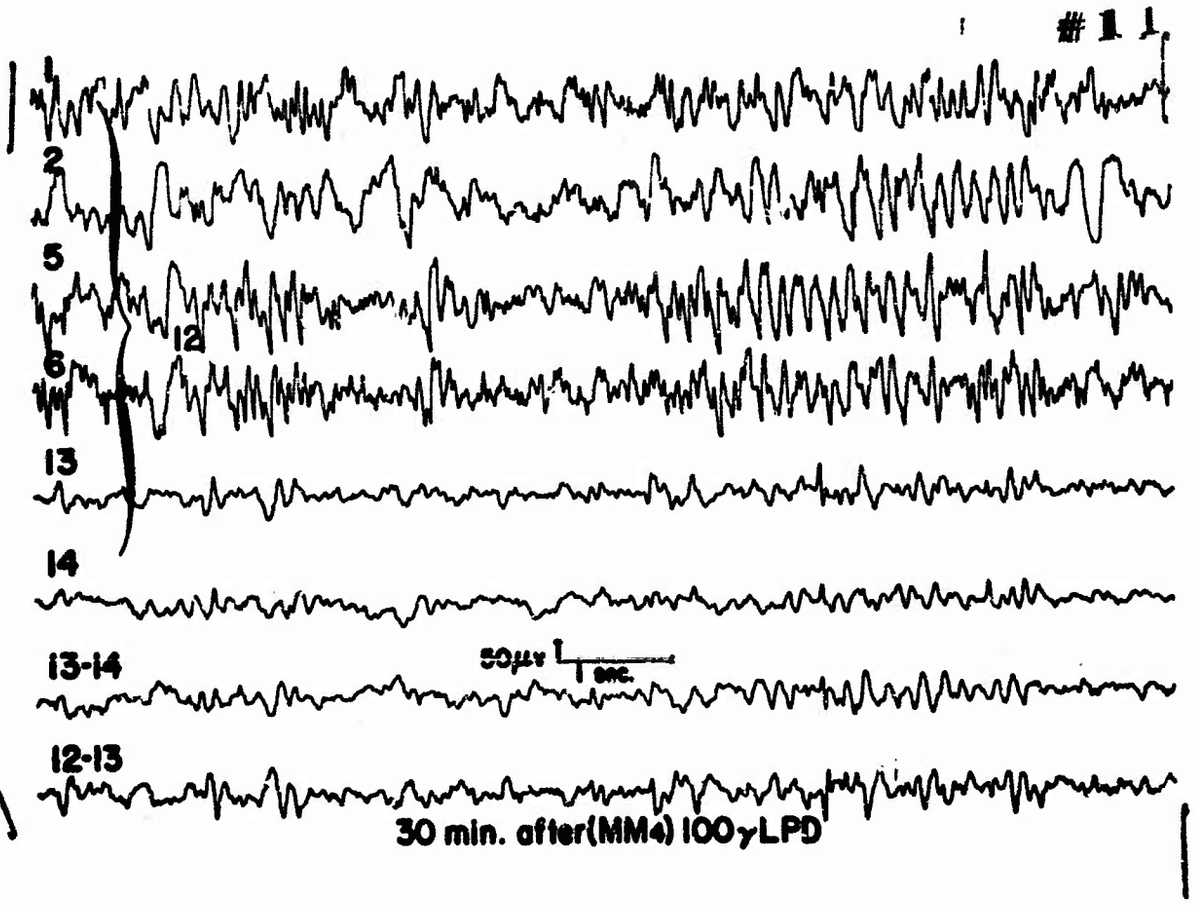


Figure 12

For baseline see Figure 13a.

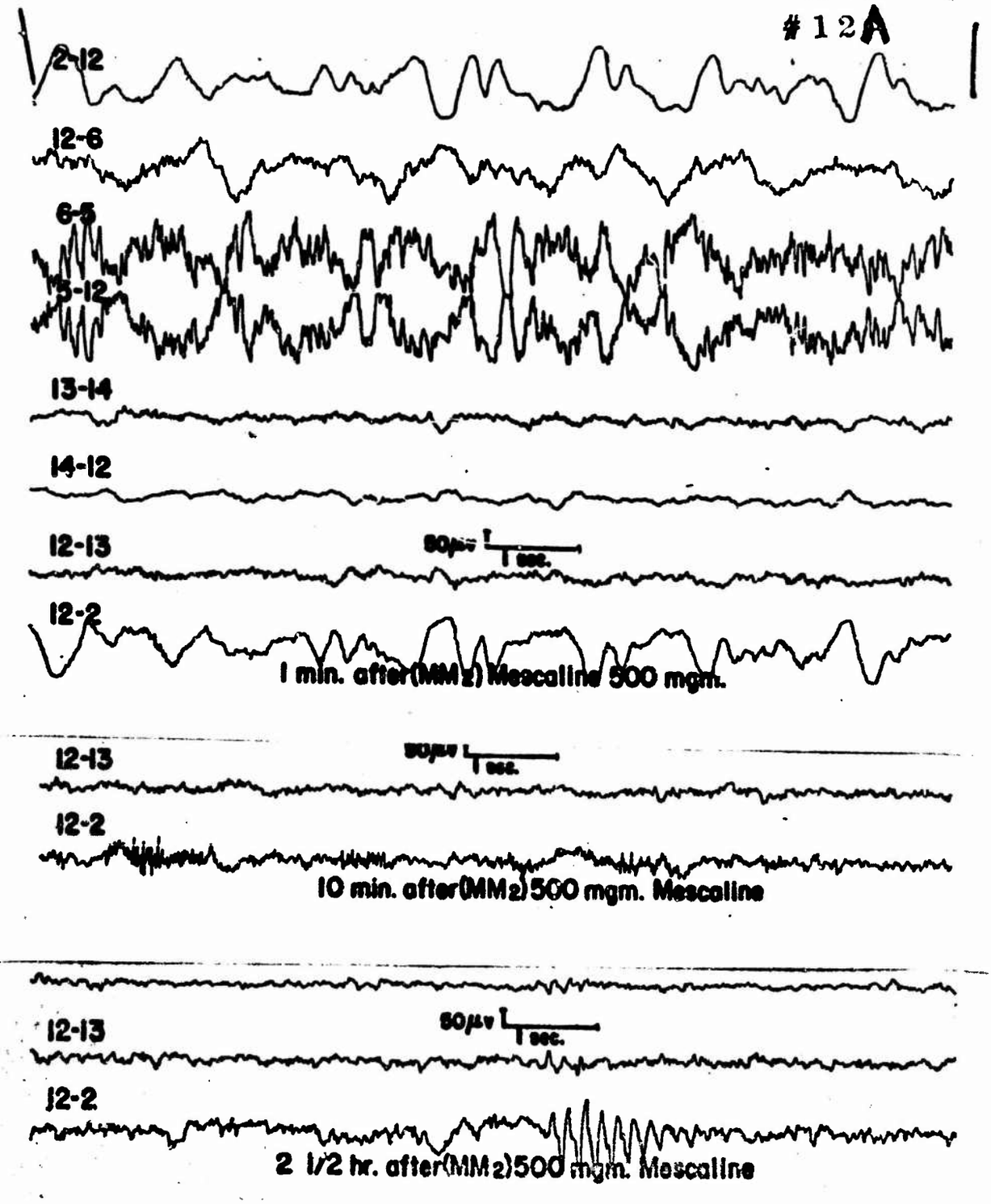


Figure 13 a & b

For explanation see text.

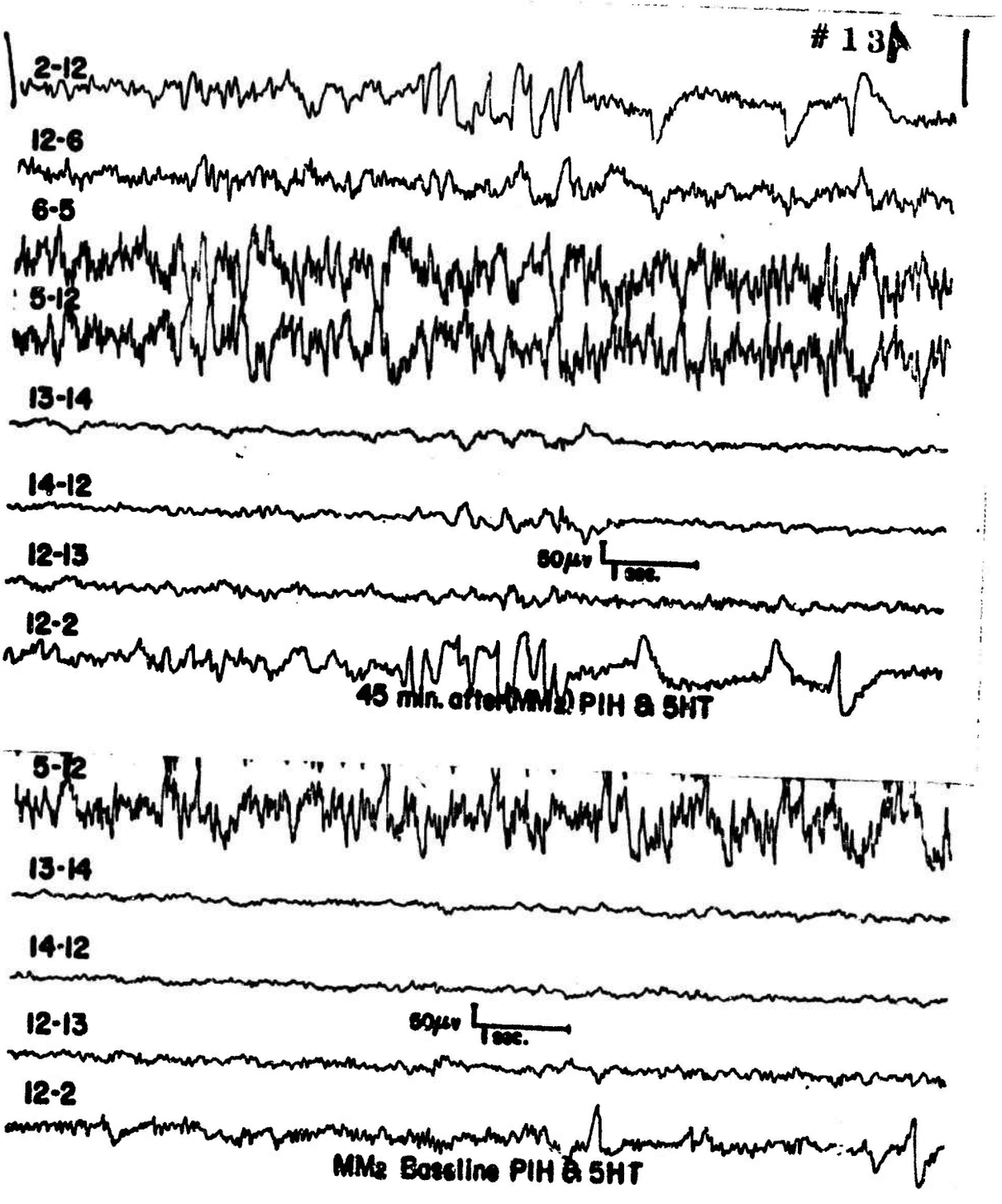


Figure 14 a, b & c

For explamation see text.